Specific PCR detection of *Pseudophaeomoniella* spp. into the xylem of healthy and *xylella fastidiosa*-infected olive trees

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Abstract: Several fungal species have been found associated with the Olive Quick Decline Syndrome (OQDS) caused by *Xylella fastidiosa* subsp. *pauca* (Xfp) in Apulia, Southern Italy. The xylem-inhabiting species, *Pseudophaeomoniella oleae* and *Ps. oleicola*, were associated with brown or black wood streaking of various olive varieties, both on young and centenarian trees. However, specific pathogenicity tests conducted on olive plantlets indicated that these fungal species have a marginal role in the aetiology of OQDS. Considering the wide distribution of *Ps. oleae* and *Ps. oleicola* over the olive-growing areas in Apulia, a genomic characterisation study was started, in order to investigate the biology of these fungi, and to ascertain any possible interaction with Xfp and the OQDS. Several polymerase chain reaction (PCR) primers were designed from the internal transcribed spacer (ITS) regions of the rDNA genes of *Pseudophaeomoniella* spp. in order to develop a species-specific detection method. Primers were screened against the two reference strains, *Ps. oleae* FV84 and *Ps. oleicola* M24, and six more *Pseudophaeomoniella* spp. isolates, resulting in the amplification of a single specific amplicon from most of the primer pairs tested. Fifteen primer pairs confirmed their specificity when tested against several isolates of different xylem-inhabiting fungal genera, such as *Phaeoacremonium*, *Pleurostomophora*, *Phaeomoniella*, *Ochroconis*, *Paraconiothydium*, *Aspergillus*, *Lophiostoma* and *Cladosporium* spp. The size of the obtained amplicons enabled the use of some primer pairs in a Real-time PCR test, using a SYBR® Green format. Results confirmed the specificity of the tested primers, thus allowing the detection and quantification of *Ps. oleae* and *Ps. oleicola*, into the xylem of both healthy and Xfp-infected olive trees. Further research is in progress to develop a specific probe for qPCR quantification of the targeted fungal species in the olive plant.

Molecular rapid detection of *Xylella fastidiosa*

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Abstract: An effective framework for early warning and rapid response is a crucial element to prevent or mitigate the impact of biological invasions of plant pathogens, especially at ports of entry. Molecular detection of pathogens by using PCR-based methods usually requires a well-equipped laboratory. Rapid detection tools that can be applied as point-of-care diagnostics are highly desirable, especially to intercept quarantine plant pathogens such as *Xylella fastidiosa*, one of the most devastating pathogens in crop and natural ecosystems. To this aim, in this study we developed a LAMP (Loop-mediated isothermal amplification) assay able to detect the target pathogen both in DNA extracted from axenic culture and in infected plant tissues. By using the portable instrument Genie ® II, the LAMP assay was able to recognise *X. fastidiosa*, DNA within 30 minutes of isothermal amplification reaction, with high levels of specificity and sensitivity. This new LAMP-based tool, allowing an on-site rapid detection of the bacterium, is especially suited to use at ports of entry, but it can also be profitably used to monitor and prevent the possible spread of the invasive pathogen in natural ecosystems.

Situation of *Xylella fastidiosa* in the Alicante outbreak of Spain and phytosanitary measures adopted

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Abstract: In June 2017, *Xylella fastidiosa* was detected in a plot of almond trees showing typical symptoms of leaf scorch in the municipality of El Castell de Guadalest (Alicante, Comunidad Valenciana, Spain). It was the first detection of the bacterium in the Iberian Peninsula (mainland Spain). Immediately, the regional Plant Health Service adopted all the phytosanitary measures in accordance with Comission Implementing Decision 2015/789 of 18 May, to prevent the introduction and spread within the EU of *X. fastidiosa*. The current demarcated area (DA), that now covers 134,581 hectares, has been increasing since the first detection due to surveillance, sampling and molecular analysis activities. In fact, in the DA during 2017 and 2018 more than 18,000 samples from different plant species have been sampled during a seasonal period favourable for the detection of the bacterium. In 2017, 201 samples out of a total of 7,650 samples, all from almond trees, were positive for *X. fastidiosa*. In 2018, 11,069 samples were taken in the DA, and *X. fastidiosa* was detected in 1,157 of them, and not only in almond trees (95.6% of the positives) but also in other species: *Polygala myrtifolia* (1.4%), *Rosmarinus officinalis* (0.3%), *Helichrysum italicum* (0.9%), *Prunus domestica* (0.3%), *Prunus armeniaca* (0.7%), *Rhamnus alaternus* (0.3%), *Calicotome spinosa* (0.2%) and *Phagnalon saxatile* (0.3%). All of these new host species were found around infected almond trees. Up to now, all the MLST analysis performed on samples from more than 39 different municipalities in the DA, in almond and other hosts, showed that only subspecies *multiplex* and the same sequence type, ST-6, is present in the DA. In addition, the Plant Health Service have also carried out periodical samplings of potential vectors, and *X. fastidiosa* has been detected in specimens of both *Philaenus spumarius* and *Neophilaneus campestris*. All specimens analysed were infected by the subspecies multiplex and ST-6 as confirmed by MLST analysis. Eradication measures have been taken since the first detection, and so far more than 40,000 almond trees have been removed and shredded according to the Comission Decision.

Public interest about *Xylella* in Italy, Europe and beyond – a spatio-temporal study

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Abstract: Google Trends is a freely available tool that makes it possible to monitor public interest in a topic, worldwide or for particular regions. Public interest is assessed on the basis of online behaviour, i.e. the number of Google queries for keywords representative of the topic of interest. This tool is increasingly used in public health as a web-based surveillance system for epidemics of human diseases. The assumption is that public interest is directly related to epidemic development. The tool (similarly to other web platforms such as Twitter and the Medisys media monitoring system) provides the potential to monitor the spatio-temporal development of public interest in plant diseases and, potentially, of plant disease epidemics too. This contribution uses Google Trends to reconstruct public interest in *Xylella fastidiosa* since 2004 in Italy, Europe and beyond. The data show the Italian, European and worldwide regions where people are most concerned by the epidemic. The tool thus provides a straightforward way for stakeholder mapping for a specific plant health threat. There is a good match between the timepoint of the peak in public interest for *Xylella* and the date of the first report of the pathogen in Italy, France, Spain and Portugal. There is thus the potential to use Google Trends for broad-scale surveillance purposes also in plant pathology. The tool also makes it possible to study public interest in various topics over time in a comparative way. For example, after the first finding of *Xylella* in Apulia in 2013, Google searches for the term 'Xylella' in Italy have become just as frequent as those for 'olive oil', whereas worldwide public interest in olive oil dwarfs that in Xylella. Taken together, these results suggest that web-based surveillance of emerging plant diseases could become another useful tool for plant health authorities, researchers and risk assessors.